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Milling and Bread Baking Traits Associated with Puroindoline Sequence Type in Hard Red Spring Wheat

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ABSTRACT

Recent results have shown that mutations in genes coding for puroindoline a and b (*PinA* and *PinB*) are associated with the expression of the hard texture of wheat (*Triticum aestivum* L.) grain. A majority of hard wheats have a glycine-to-serine mutation in puroindoline b (allele *PinB-D1b*), or they are devoid of puroindoline a (allele *PinA-D1b*). Hard wheats with *PinA-D1b* tend to be harder than those with *PinB-D1b*. Grain hardness is known to affect milling and baking traits. Our objective was to determine the influence of allelic variation in *PinA* and *PinB* on milling and bread quality traits in a recombinant inbred population segregating for *PinA-D1b* and *PinB-D1b*. One hundred thirty-nine recombinant inbred lines from the cross 'Butte 86' (*PinA-D1b* allele)/ND2603 (*PinB-D1b* allele) and parents were grown in a field trial with two replications at two locations. Grain hardness was measured by near-infrared reflectance (NIR) and the single-kernel characterization system (SKCS). Grain was milled and baked for each line. Puroindoline allele type was determined for each line. The *PinB-D1b* group had significantly softer grain, higher break flour yield, flour yield, milling score, and loaf volume, and lower flour ash and crumb grain score (low score being desirable) than the *PinA-D1b* group. Significant genetic variability was detected within allelic classes for all traits. The proportion of variation among entry means attributed to puroindoline classes was 34% for break flour yield, 26% for NIR hardness, and 22% for SKCS hardness index. Grain hardness was negatively correlated with break flour yield, flour yield, and mixing score and positively correlated with flour ash. Grain hardness was not correlated with loaf volume or crumb grain score. The *PinB-D1b* allele was more desirable for milling and bread baking, although superior milling and bread quality genotypes could be selected within either class.

WHEAT IS CLASSIFIED into hard and soft classes on the basis of the texture of the grain. These textural

classes coincide with differences in milling and end-use properties (reviewed in Pomeranz and Williams, 1990; Morris and Rose, 1996). The distinction between soft and hard classes of wheat is governed by the Hardness (*Ha*) locus on chromosome 5DS (Mattern et al., 1973; Law et al., 1978) with additional modifying genes contributing to variation within classes (Symes, 1965; Baker, 1977); however, Baker and Sutherland (1991) and Giroux et al. (2000) observed significant genetic variation for grain hardness within crosses of hard wheats.

Greenwell and Schofield (1986) identified friabilin as a marker protein for grain softness which was present in larger amounts on the surface of water-washed starch of soft wheats than from hard wheats (Bettge et al., 1995; Greenblatt et al., 1995; Morris et al., 1994). Friabilin is composed of two major polypeptides termed puroindoline a and puroindoline b. Genes coding for these two proteins, *PinA* and *PinB*, are tightly linked to the *Ha* locus on chromosome 5D (Jolly et al., 1993; Sourdille et al., 1996) and probably function together as the *Ha* locus. Recent results have shown that mutations in *PinA* and *PinB* are associated with the expression of hard texture. Giroux and Morris (1997, 1998) showed that hard texture was completely linked to a glycine-to-serine mutation in puroindoline b (allele *PinB-D1b*), or the complete absence of the puroindoline a protein (allele *PinA-D1b*). In a survey of hard wheats, cultivars with the *PinA-D1b* allele were on average 7 units harder than those with *PinB-D1b* (Giroux and Morris, 1997; unpublished results). Giroux et al. (2000) further showed that progeny carrying the *PinA-D1b* allele averaged 4.5 units harder than progeny with *PinB-D1b* in three hard red spring crosses segregating for *PinA-D1b* vs *PinB-D1b*. A more recent survey has found additional mutant alleles of *PinA* or *PinB* linked to hard textured grain (Lillemo and Morris, 2000).

Since kernel texture has been shown to be associated with numerous milling and bread quality traits in hard wheats (Slaughter et al., 1992) and Giroux et al. (2000) showed hard wheats with the *PinA-D1b* allele tend to be harder than those with the *PinB-D1b* allele, it is possible that allelic variation at the *PinA* and *PinB* loci

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Table 1. Puroindoline mutation class means, heritability, range and parent means for test weight, grain hardness, kernel morphology traits, and protein concentration for recombinant inbred lines from Butte 86 (*PinA-D1b* allele) × ND2603 (*PinB-D1b* allele) spring wheat cross based on mean of two locations.

Entry	Test weight	Grain hardness		Kernel weight	Kernel diameter	Wheat protein	Flour protein
		NIR†	SKCS‡				
	kg m ⁻³			mg	mm	g kg ⁻¹	
<i>PinA-D1b</i>	772	81.9	64.3	35.3	2.45	149	133
<i>PinB-D1b</i>	769	74.1	58.3	34.9	2.41	148	133
		**	**				
Heritability	0.55	0.70	0.88	0.59	0.71	0.79	0.84
Range	662–802	58.7–97.5	41.8–73.6	28.9–44.6	2.10–2.83	130–170	115–157
Butte 86	768	81.5	63.8	32.3	2.36	149	135
ND 2603	778	72.5	61.0	35.4	2.40	139	125
LSD	30	11.5	5.8	3.5	0.18	11	6
CV%	2.4	6.8	3.6	3.1	2.8	3.7	3.1

** Difference between puroindoline class means is significantly different from zero at the 0.05 and 0.01 probability levels, respectively.

† Near-infrared reflectance.

‡ Single kernel characterization system.

affects milling and bread quality traits. Our objective was to determine the influence of allelic variation in *PinA* and *PinB* on milling and bread quality traits in a recombinant inbred population segregating for *PinA-D1b* and *PinB-D1b*.

MATERIALS AND METHODS

One hundred thirty-nine hard red spring wheat recombinant inbred lines were derived from the cross 'Butte 86'/ND 2603. Lines were derived by single-seed-descent from F₂ to F₆ followed by a generation of seed increase to produce F₆-derived F₈ lines. Butte 86 carries the *PinA-D1b* allele while ND 2603 has the *PinB-D1b* allele. The ND 2603 parent has pedigree 'Wheaton'/'Sumai 3'. The 139 recombinant inbred lines and parents were grown at Pullman, WA, and 134 lines and parents were grown at Bozeman, MT, in 1998. Each trial was a randomized complete block design with two replications. Each plot was a single 3-m row with 30 cm between rows. Grain from each plot was threshed for milling and bread quality analyses.

Wheat samples were analyzed for moisture content (Method 44-16) and test weight (Method 55-10) (AACC, 2000). Near-infrared reflectance (NIR) hardness (Method 39-70A) (AACC, 2000) was determined from a single aliquot using a near-infrared reflectance spectrometer (model IA450, Technicon, Hoganas, Sweden) on whole grain meal (0.5-mm screen) (UDY Corp., Fort Collins, CO). The Single Kernel Characterization System 4100 (SKCS) (Perten Instruments North America, Inc., Springfield, IL) was used to estimate grain hardness (SKCS hardness index), kernel weight, and kernel diameter (thickness or outer diameter) using a sample of 300 kernels from each plot.

Wheat was tempered to 145 g kg⁻¹ (14.5%) moisture (fresh weight basis) and milled on Quadrumat Jr. mills following modifications of Jeffers and Rubenthaler (1977). Mill output was separated using 35- (500 µm) and 100-mesh (150 µm) Tyler test sieves into bran, middlings stock and break flour fractions. Break flour yield was calculated as the proportion of break flour to total products. Middlings were further milled to produce reduction flour and shorts. All tests were conducted on "straight-grade" flour derived from combining the break and reduction flour streams, and expressed as flour yield as a proportion of total products. Milling score was calculated as:

$$\begin{aligned} & \{ [100 - [0.50 \times (16\text{-percent temper level}) \\ & + (80 - \text{flour yield}) + 50 \\ & \times (\text{percentage flour ash} - 0.30)] \} \\ & \times 1.045 \} - 3.438. \end{aligned}$$

Nitrogen content of wheat and flour was determined on a 0.25-g aliquot of the UDY-ground sample using the Dumas combustion method (model FP-428, Leco Corp., St. Joseph, MI) (AACC, 2000), and converted to percentage protein by multiplying by 5.7. Flour was analyzed for moisture (Method 44-16), protein (Method 46-30), and ash (Method 08-01) (AACC, 2000). All grain and flour parameters are reported on a 120 g kg⁻¹ (12%) and 140 g kg⁻¹ (14%) moisture basis, respectively. Mixogram analysis was conducted using the 10-g instrument following Method 54-40A (AACC, 2000).

Bread was baked and scored according to Method 10-10B (AACC, 2000) using an optimum absorption, optimum mixing, 90-min fermentation "straight-dough" comprised of 100 g flour (14% mb), 1.8 g dry active yeast, 1.5 g NaCl, 6 g sucrose,

Table 2. Puroindoline mutation class means, heritability, range and parent means for milling and bread quality traits for recombinant inbred lines from Butte 86 (*PinA-D1b* allele) × ND2603 (*PinB-D1b* allele) spring wheat cross based on mean of two locations.

Entry	Flour yield	Break flour yield	Milling score†	Flour ash	Mixograph absorption	Bake absorption	Mix time	Loaf volume	Crumb grain score‡
	g kg ⁻¹	g kg ⁻¹			g kg ⁻¹		min	ML	
<i>PinA-D1b</i>	661	387	80.0	4.09	657	682	2.78	970	4.43
<i>PinB-D1b</i>	674	430	82.4	3.90	656	680	2.86	995	3.86
	**	**	**	**				*	*
Heritability	0.78	0.85	0.83	0.72	0.59	0.63	0.85	0.55	0.71
Range	626–706	337–484	74.3–87.8	3.47–5.20	614–691	634–716	1.23–4.65	752–1155	2.00–8.50
Butte 86	683	437	82.5	4.07	654	676	3.54	968	4.02
ND 2603	685	433	84.0	3.82	654	682	3.05	937	4.45
LSD	23	37	2.9	0.40	20	20	0.68	117	2.0
CV%	1.4	4.0	1.7	5.5	2.2	2.1	9.8	6.8	24.8

*, ** Difference between puroindoline class means is significantly different from zero at the 0.05 and 0.01 probability levels, respectively.

† Milling score was calculated as: $\{ [100 - [0.50 \times (16 - \text{percent temper level}) + (80 - \text{flour yield}) + 50 \times (\text{percent flour ash} - 0.30)] \} \times 1.045 \} - 3.438$.

‡ Based on 1 (excellent) to 9 (unsatisfactory) scale.

Table 3. Correlations between grain hardness and milling and bread baking traits for 131 recombinant inbred lines from Butte 86 (*PinA-D1b* allele) × ND2603 (*PinB-D1b* allele) spring wheat cross based on mean of two locations.

Variable	Near-infrared reflectance hardness	Single kernel characterization system hardness index
Test weight	0.11	0.11
Kernel weight	-0.07	-0.28**
Kernel diameter	0.02	-0.21*
Wheat protein	0.18*	-0.40**
Flour yield	-0.24**	-0.54**
Break flour yield	-0.55**	-0.77**
Flour ash	0.45**	0.30**
Milling score	-0.40**	-0.58**
Flour protein	0.19*	-0.47**
Mixograph absorption	0.24**	-0.05
Bake absorption	0.30**	0.04
Mix time	-0.06	0.24**
Loaf volume	-0.14	-0.15
Crumb grain score	0.15	0.09

*, ** Significantly different from zero at the 0.05 and 0.01 probability levels, respectively.

0.3 g malt extract (60 mg commercial malted barley flour [Amylomalt, Cargill Flour Milling, Ogden, UT] per mL extract), 4 g powdered nonfat dry milk, 3 g partially hydrogenated vegetable shortening with mono- and diglycerides (Crisco, Procter & Gamble, Cincinnati, OH), and 7.5 mg ascorbic acid. Crumb grain was scored on the basis of the consensus score of three experienced bakers using a range of 1 (excellent) to 9 (unsatisfactory).

Puroindoline allele type was determined as previously described (Giroux and Morris, 1998). Briefly, Triton X-114 solu-

Table 4. Correlations among milling and bread quality traits where *PinA-D1b* lines differed from *PinB-D1b* lines for 131 recombinant inbred lines from Butte 86 (*PinA-D1b* allele) × ND2603 (*PinB-D1b* allele) spring wheat cross segregating for the two alleles based on mean of two locations.

Trait	Flour yield	Break flour yield	Flour ash	Milling score	Loaf volume
Break flour yield	0.81**				
Flour ash	-0.25*	-0.42**			
Milling score	0.87**	0.81**	-0.70**		
Loaf volume	0.21*	0.26**	-0.09	0.20*	
Crumb grain score	-0.31**	-0.32*	0.16	-0.32**	-0.77**

*, ** Significantly different from zero at 0.05 and 0.01 probability level, respectively.

ble proteins were extracted from a sample of 10 to 20 seeds and then subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). Samples were screened for the presence or absence of puroindoline a. Presence of puroindoline a was interpreted as *PinB-D1b* allele (the serine mutation in *PinB*) and absence as *PinA-D1b* allele (devoid of puroindoline a).

Analyses of variance combined across locations were performed for each trait, where locations were considered fixed and replications within locations, entries, and entry × location interaction as random effects. Heritability estimates were computed on a progeny mean basis for each trait. The entry variation was further partitioned by including a fixed effect for puroindoline class and a random effect for entries within class and associated interactions with location. The analyses were

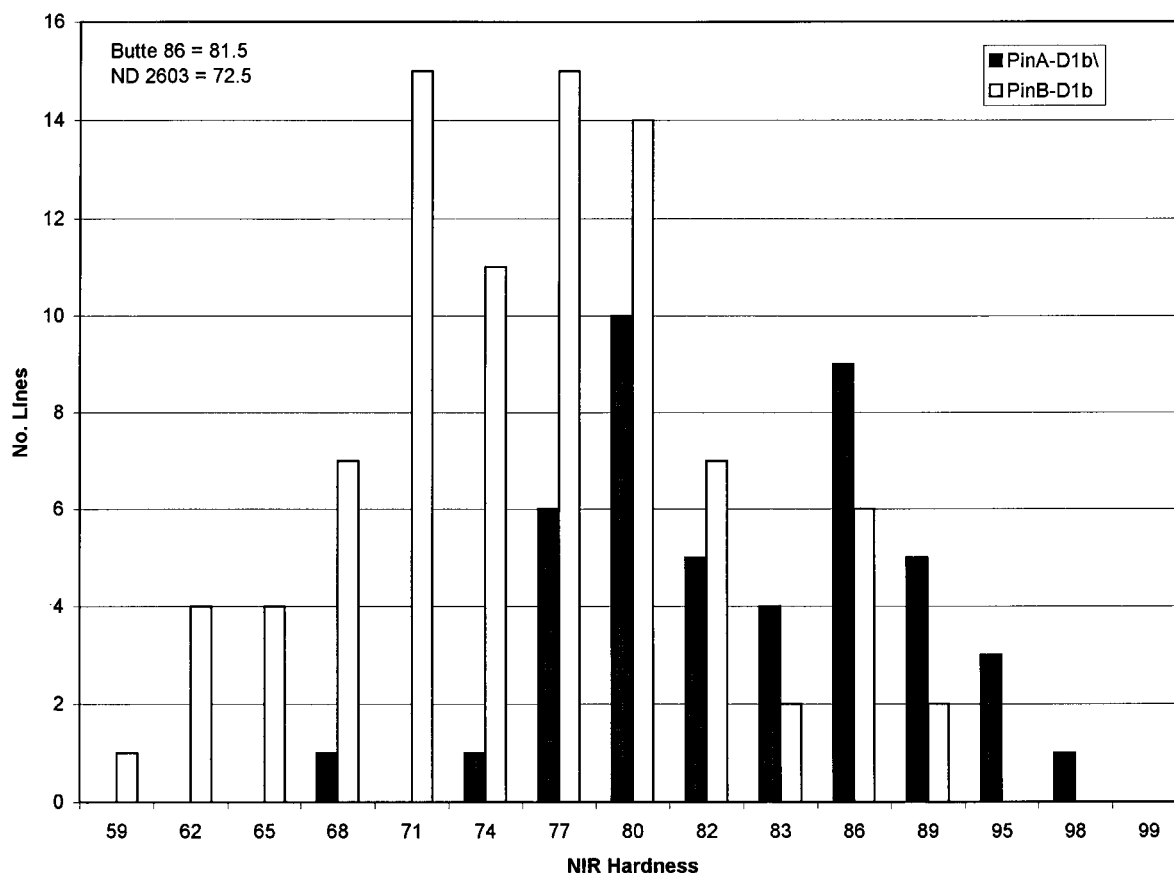


Fig. 1. Distribution of 45 lines within *PinA-D1b* class and 89 lines within *PinB-D1b* class for Near-infrared reflectance (NIR) hardness from a cross between Butte 86 (*PinA-D1b* allele) and ND 2603 (*PinB-D1b* allele) determined on the basis of mean of two locations.

carried out by PROC MIXED in SAS (SAS Institute, 1997). Least squares means were obtained for the two puroindoline classes, and the difference between the *PinA-D1b* and *PinB-D1b* means was compared with a *t*-statistic, where the standard error of the difference was computed from the appropriate linear combination of mean squares and degrees of freedom by the Satterthwaite (1946) approximation. Transgressive segregates were defined as those lines that were less than the lowest ranking parent or greater than the highest ranking parent by more than one LSD. The proportion of the variation attributed to the *PinA-D1b* vs *PinB-D1b* class difference was determined as the ratio of the class sum of squares to the entry sum of squares. Correlations among traits were computed from entry means. Three lines at the Bozeman location did not provide enough flour for baking. These lines were dropped from analyses for bake absorption, loaf volume, and crumb grain score.

RESULTS AND DISCUSSION

Significant genetic variation was observed for all traits. Entry \times location interaction variances were also significant in all instances. However, the location \times puroindoline mutation class interaction was not significant for any trait.

The two parents could not be differentiated statistically for any of the traits except flour protein where Butte 86 (*PinA-D1b*) exceeded ND 2603 (*PinB-D1b*) (Table 1). There was a trend for Butte 86 to have harder kernels (81.5 vs 72.5 NIR units and 63.8 vs 61.0 SKCS

units), higher grain protein (149 vs 139 g kg⁻¹), higher flour ash (4.07 vs 3.82 g kg⁻¹), and longer mixing time (3.54 vs 3.05 min), but lower kernel weight (32.3 vs 35.4 mg kernel⁻¹) than ND 2603.

Narrow sense heritability estimates ranged from 0.55 for test weight and loaf volume to 0.88 for SKCS hardness index (Tables 1 and 2). All traits showed transgressive segregation in both directions except test weight and total flour yield, for which transgressive segregation was only negative, and kernel weight and flour ash which showed only positive transgressive segregants. Campbell et al. (1999) found transgressive segregation for kernel texture, kernel morphological traits, and milling and cookie quality traits (flour yield, softness equivalent, alkaline water retention capacity, and cookie diameter) (K. G. Campbell, 2000, personal communication) in a soft \times hard wheat cross.

The 139 recombinant inbred lines segregated 47 *PinA-D1b*: 92 *PinB-D1b* which deviated significantly ($P < 0.01$) from the expected 1:1 ratio. Genes coding for puroindoline a and b proteins are tightly linked on chromosome 5DS. Other reports with populations segregating for *PinA-D1b* and *PinB-D1b* also have reported distorted segregation ratios (Giroux and Morris, 1997; Giroux et al., 2000). The *PinA-D1b* lines exceeded the *PinB-D1b* lines by 9 NIR hardness units and 6 SKCS hardness units (Table 1). This difference is in agreement with the 7 hardness unit difference Giroux (personal

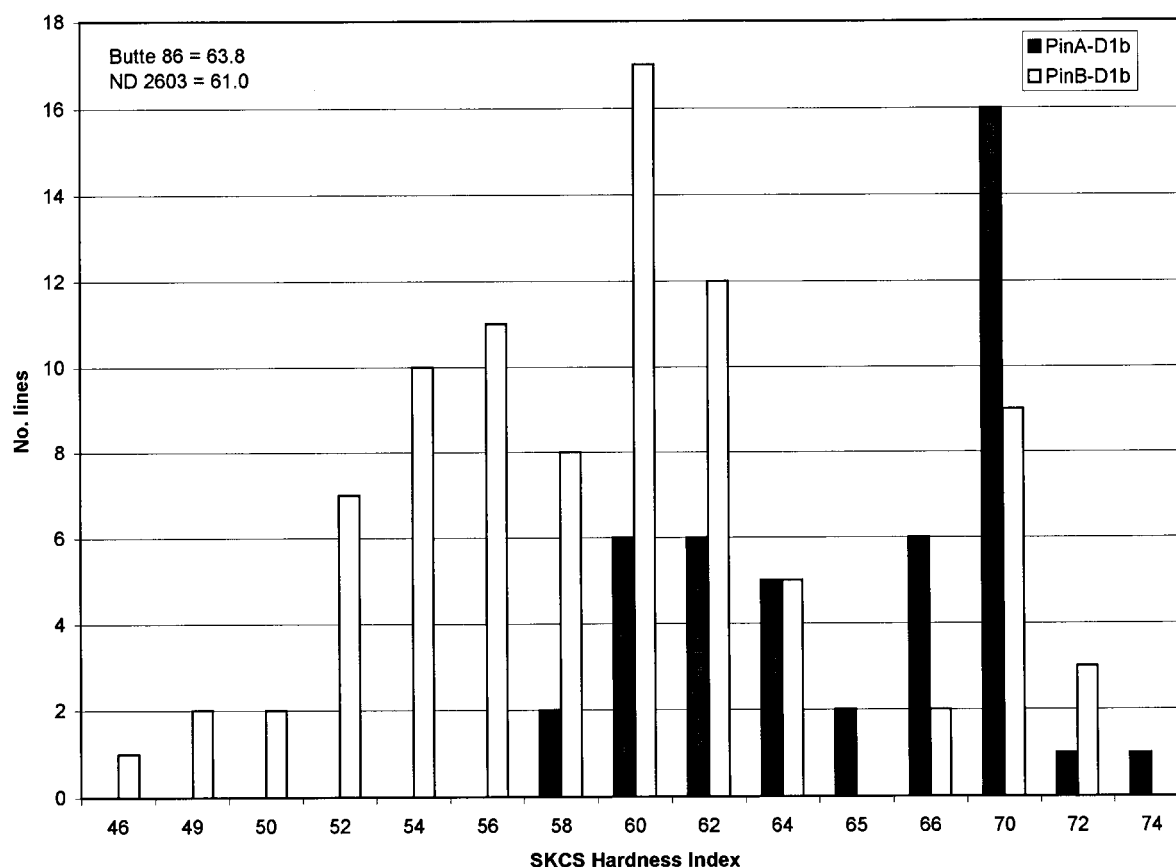


Fig. 2. Distribution of 45 lines within *PinA-D1b* class and 89 lines within *PinB-D1b* class for single kernel characterization system (SKCS) hardness index from a cross between Butte 86 (*PinA-D1b* allele) and ND 2603 (*PinB-D1b* allele) determined on the basis of mean of two locations.

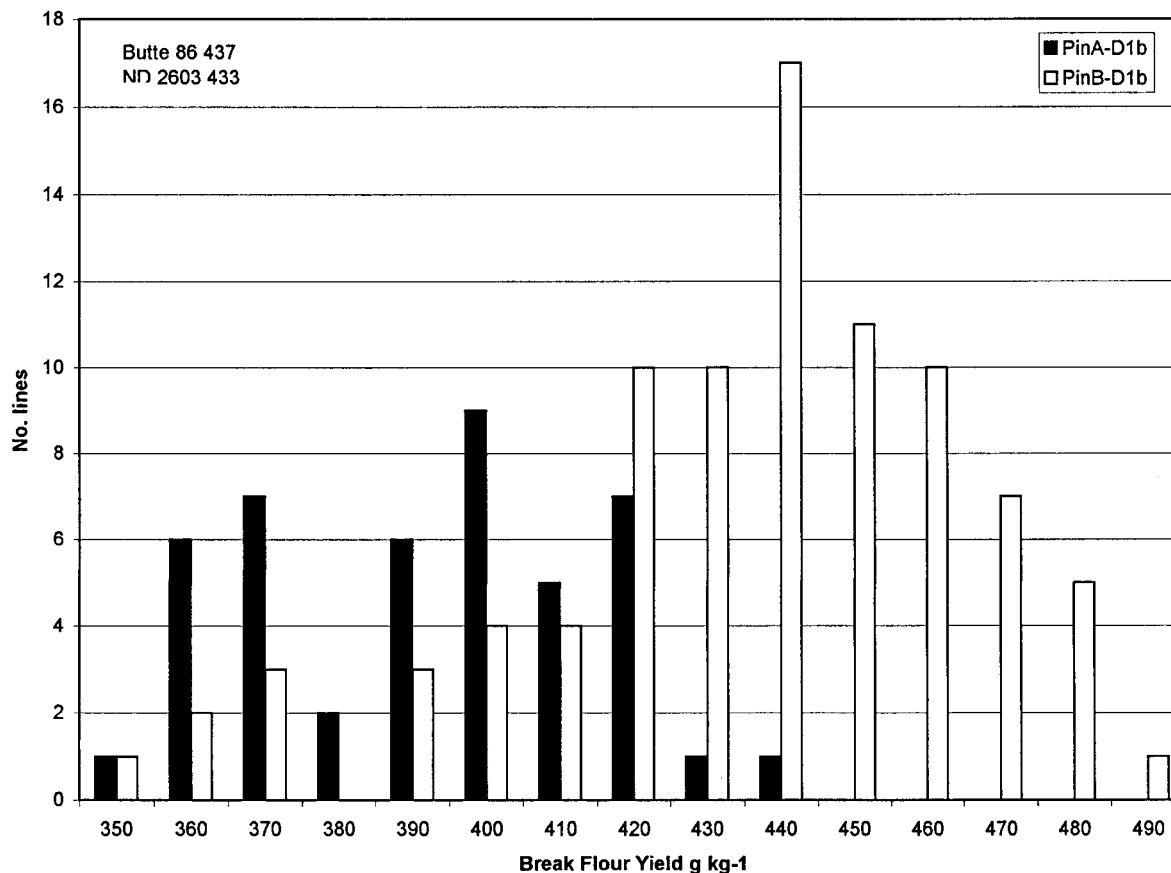


Fig. 3. Distribution of 45 lines within *PinA-D1b* class and 89 lines within *PinB-D1b* class for percentage break flour yield from a cross between Butte 86 (*PinA-D1b* allele) and ND 2603 (*PinB-D1b* allele) determined on the basis of mean of two locations.

communication, 1999) reported from a survey of hard wheats. Difference in hardness between the two classes is greater than the 4.5 hardness unit difference measured for three hard red spring wheat crosses segregating for *PinA-D1b* and *PinB-D1b* (Giroux et al., 2000). They may have underestimated the hardness difference between the two alleles, since hardness was measured on whole grain using near-infrared-transmission. The softer textured *PinB-D1b* group had significantly higher flour yield (674 vs 661 g kg⁻¹) and break flour yield (430 vs 387 g kg⁻¹), milling score (82.4 vs 80.0), and loaf volume (995 vs 970 mL), and lower flour ash (3.90 vs 4.09 g kg⁻¹) and crumb grain score (3.84 vs 4.44) than the harder textured *PinA-D1b* group (Table 2). These differences were consistent in both locations except for loaf volume and crumb grain score where the difference was significant ($P < 0.05$) at Pullman, but was not statistically different for Bozeman (0.14 and 0.13 probability levels, respectively). The proportion of variation among entry means attributed to the difference between *PinA-D1b* and *PinB-D1b* was 34% for break flour yield, 26% for NIR hardness, 22% for SKCS hardness index, and 17% for milling score. This allelic difference explained less than 11% of the variation for the remaining traits, with significant genetic variation within the two classes observed for all traits. The large effect of this allelic difference on indices of grain hardness is striking in that both parents are hard textured, and were similar in

phenotypic expression for these indices (Tables 1 and 2). The *PinB* locus segregating in a soft \times hard wheat cross accounted for more than 60% of the variation in kernel texture (Campbell et al., 1999) and had an effect larger than other marker loci for milling and cookie traits (K.G. Campbell, 2000, personal communication).

The NIR and SKCS methods of measuring grain hardness were correlated ($r = 0.53$; $P < 0.01$). That is less than the $r = 0.87$ ($P < 0.01$) between the two methods Morris et al. (1999) reported using 83 recombinant chromosome 5D substitution lines segregating for soft vs hard grain texture for 5D and $r = 0.81$ ($P < 0.01$) for 72 hard wheat samples (Ohm et al., 1998). Both methods were positively correlated ($P < 0.01$) with flour ash but negatively correlated ($P < 0.01$) with break flour yield, flour yield, and milling score (Table 3). NIR hardness was positively correlated with mixograph and bake absorption, while SKCS hardness index was not correlated with either. Conversely, SKCS hardness index was negatively correlated with kernel weight ($P < 0.01$) and kernel diameter ($P < 0.05$), but NIR hardness was not correlated with either. The greatest disparity between the two methods occurred for wheat and flour protein concentration where NIR hardness was positively correlated ($P < 0.05$) with both, but SKCS hardness index was negatively correlated ($P < 0.01$) with both. Wheat protein is often positively related with grain hardness within hard wheats (Giroux et al., 2000; Slaughter et

al., 1992). The less than complete association between NIR and SKCS hardness index, and their different associations with some traits may reflect the differing approaches to quantifying grain hardness. NIR estimates hardness through spectral characteristics of particle size distribution. SKCS hardness index results from a force-deformation curve derived from crushing individual kernels that is influenced by moisture, kernel size, and kernel weight (Martin et al., 1993).

NIR hardness, SKCS hardness index, and break flour yield all have been used to quantify grain hardness (Morris et al., 1999) and the three traits were highly interrelated (Tables 3 and 4). The distribution of lines within *PinA-D1b* and *PinB-D1b* showed similar patterns for NIR hardness (Fig. 1), SKCS hardness index (Fig. 2), and break flour yield (Fig. 3). The distribution of lines within the *PinB-D1b* group completely overlapped that for the *PinA-D1b* group. The wider range observed for the *PinB-D1b* group was in part due to the larger sample size. Extreme values within the two groups could represent recombinant types, since we assayed for *PinA-D1b* and assumed the remainder to be *PinB-D1b*. This seems unlikely, as recombination between *PinA* and *PinB* genes has not been observed in other populations (Giroux and Morris, 1997). Tranquilli et al. (1999) reported *PinA* and *PinB* were tightly linked with 0.14 centimorgans between them.

Alterations in the *PinA* and *PinB* loci had greatest influence on traits related to particle size and subsequent milling properties of the grain, namely grain hardness, break flour yield, flour yield, milling score, and flour ash. It had a smaller effect on loaf volume and crumb grain score (Tables 1 and 2). These traits were highly correlated among themselves (Table 4) except that grain hardness measures were not associated with loaf volume or crumb grain score (Table 3). All were positively associated, except flour ash and crumb grain score were inversely associated with the other traits.

Hard wheats suffer more starch damage during milling and tend to absorb more water during dough formation than soft wheats (Slaughter et al., 1992). Surprisingly, the harder textured *PinA-D1b* group did not differ from the *PinB-D1b* group for mixograph or bake absorption (Table 2). In addition, hardness was only weakly associated with absorption ($r < 0.3$). This may suggest that our genotypes represented a smaller range in hardness than would be encountered in comparing hard and soft textured wheats.

Giroux and Morris (1997, 1998) observed that hard wheats had either the *PinB-D1b* allele (the serine mutation in *PinB*) or the *PinA-D1b* allele (devoid of puroindoline a). Our results and those from Giroux et al. (2000) have confirmed that *PinA-D1b* genotypes are harder than *PinB-D1b* genotypes. Dubreil et al. (1998) observed that puroindoline a concentration was inversely related to grain hardness in a sample of 32 wheats. The softer grain conferred by *PinB-D1b* may be a function of quantity of puroindoline a, b, or both. Some residual function of *PinB* may remain in *PinB-D1b* types versus the more severe mutation in *PinA-D1b*. Dubreil et al. (1998) also observed that flours that were opposite in

bread quality, but lacking puroindoline a, had significantly higher loaf volumes than the same flours reconstituted with puroindolines (80% puroindoline a and 20% puroindoline b). Rheological properties of dough were altered in opposite directions by addition of puroindolines. Dough strength and extensibility were reduced in the poor quality flour but increased when puroindolines were added to the good quality flour. Our results showed lower loaf volume from flours lacking puroindoline a (*PinA-D1b* allele) compared to those with both puroindoline a and b (*PinB-D1b* allele). These findings, coupled with those from Dubreil et al. (1998) suggest that quantity of puroindolines and/or the ratio of puroindoline a to b may have a role in dough formation and resultant loaf volume.

If the *PinA-D1b* or *PinB-D1b* allele conferred an advantage for improved milling or bread quality traits, breeders could use it as a selectable marker to improve milling or baking quality. Our results showed that the *PinB-D1b* allele may be more desirable because it conferred a significant advantage over the *PinA-D1b* allele through increased break flour and flour yield, milling score, and loaf volume with lower flour ash and crumb grain score (lower crumb grain score being desirable). Significant genetic variability was detected within allelic classes for all traits, indicating that superior milling and bread quality genotypes could be selected within either class.

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Core Collection of Sorghum: I. Stratification Based on Eco-Geographical Data

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ABSTRACT

ICRISAT conserves a large (36 719 entries) collection of sorghum [*Sorghum bicolor* (L.) Moench] accessions in India. This collection comprises cultivated and wild sorghums acquired over the past 25 years from 90 countries. However, it is difficult to characterize and assess a large collection with limited time and resources. To facilitate maintenance, assessment, and utilization of the collection, we considered the establishment of a core collection using stratified sampling strategies. Results from a study of the morpho-agronomic diversity were used to describe the genetic structure of the collection. Morphological traits, including days to flowering and plant height, can be affected by daylength variation. These two characters were highly correlated with latitudinal and racial distributions of landraces. Thus, stratifying the entire collection for response to photoperiod, estimated by flowering date and plant height, was indicative of a major source of specific adaptation within the collection. This stratification resulted in four clusters, which described the sensitivity of genotypes to photoperiod within the latitudinal range where selection was carried out by farmers. These four clusters may serve as the basis for a random stratified sampling to establish cores in this collection.

SORGHUM is the fifth most important cereal crop in the world based on total grain production and is an essential component of the cropping systems of subsis-

tence farmers and the diets of millions of people in the semiarid tropics. The continued improvement of this important crop depends upon the utilization of genetic variability in landraces originally maintained by traditional agricultural practices. Taxonomically, *Sorghum bicolor* (L.) Moench subs. *bicolor* has 15 races: the 5 basic races of bicolor, durra, caudatum, kafir, and guinea, and their 10 intermediates (Harlan and de Wet, 1972). The large set of cultivated sorghums, presently maintained at ICRISAT Asia Center (IAC), Patancheru, India, has been assembled from 40 countries in Africa, 24 in Asia, 11 in Europe, 13 in the Americas, and several entries from Russia and Australia.

To facilitate the use of such a large collection, establishing core collections is recommended in order to prioritize maintenance and evaluation on subsets that retain a large part of the diversity encompassed in the entire collection (Brown, 1989a). Different sampling strategies are proposed including sampling based on random procedures applied on a stratified collection (Brown, 1989b). The choice of criterion to stratify the collection depends on the data set available and the objectives for establishing a core. Several studies have shown that phenotypic divergence among and within landrace populations is related to geographical distance between countries of origin (Spagnoletti Zeuli and Qualset, 1987; Peeters and Martinelli, 1989; Schoen and Brown, 1993; Spagnoletti Zeuli and Qualset, 1993).

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